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(54) Organism carrying a Single Chain Antibody Domain at its surface.

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Description

The present invention relates to the production of genetically engineered organisms and methods of preparation of binding molecules.

In vertebrates, antibody diversity arises from substitution of hypervariable loops into constant antibody frame-works. Each B-cell exhibits its own type of specificity on its surface. When an antigen binds to the surface antibody, the B-cell is stimulated to proliferate.

Monoclonal antibody production exploits this as follows: An animal is injected with a purified antigen. After several weeks, the spleen is removed from the animal and spleen cells are fused to myeloma cells. This produces hybridoma cells. These cells are plated and screened for binding to antigen. These cells can be grown in tissue culture and will produce quantities of a single antibody--a monoclonal antibody.

The gene for the antibody can be recovered and put into microorganisms. Genetic and protein engineering can be altered to obtain better binding, altered specificity, different antigenic behavior than that of the original protein or gene product.

Single-chain antibodies (SCA) (referred to in co-pending U.S. Patent Application Serial Nos. 902,971 and 902,970) are protein molecules which retain the binding domain of antibodies but not the effector domains.

A. Klausner, ***Biotechnology*, 4:** 1041-1043 (1986); refers to the production of single chain antibodies in *E. coli*.

US-A-4603112, US-A-4593002 and P. Valenzuela *et al*, ***Biotechnology*, 3:** 323-326 (April 1985) all refer to the expression of immunogens on the surface of viruses for use as vaccines.

Summary of the Invention

Thus according to the invention, there is provided an organism containing a recombinant gene wherein the product of the recombinant gene is a fusion of a polypeptide normally appearing on the outer surface of the organism with a single chain antibody domain (SCAD).

The invention also extends to a fusion polypeptide comprising a product normally appearing on the surface of an organism fused to a single chain antibody domain (SCAD).

The invention additionally extends to a method of preparing an organism containing a single chain binding molecule on the outer surface of the organism which comprises:

(1) isolating from an organism a first gene encoding for a cell polypeptide normally appearing on the surface of the organism;

(2) inserting a second gene which encodes a single chain antibody domain into the first gene to form a recombinant fusion gene; and

(3) transforming an organism with the recombinant fusion gene.

Thus in the present invention, a genetically engineered organism is produced which displays on the outer surface of the organism the expression product of a gene which has been inserted. In one embodiment a SCA domain (SCAD) is displayed on the outside of a microorganism while the message for that particular SCA is inside that organism.

Description of the Drawings

Figure 1, flow chart for production of organisms containing binding molecules on surface.

Figure 2, displaying SCAD on surface.

Figure 3, making diverse population of displayed SCADs.

Figure 4, selecting new SCA specificity.

Figure 5, detecting known antigens.

Figure 6, lambda assembly.

Figure 7, inserting SCAD into V genes.

DESCRIPTION OF PREFERRED EMBODIMENTS

Any protein or antibody domain for which a gene can be isolated or constructed may be displayed on the outer surface of an organism into which the gene has been inserted. This is done by fusing the SCAD gene to the gene coding for a product which normally expresses on the surface of the organism; e.g., an envelope protein. The organism so produced may be easily isolated from organisms which do not contain the desired gene and express the gene product. The organisms may also serve as a solid substrate for the

gene product. Prior to the present invention, once an organism which contained a desired gene had been produced, the organisms had to be grown and assayed for the production of the gene product. Then, the gene product had to be isolated, purified, and only then was it possible to couple it to a solid substrate. In one embodiment, the organism itself containing the gene product on its outer surface is the solid substrate with the desired gene product already attached and may be used as such.

The present invention is depicted in general terms in the flow chart on Figure 1. One embodiment shown at step 1000 consists of producing organisms. In this embodiment, a microorganism displays a gene product such as a SCAD on the surface of the organism. The next step (step 1010) consists of generating, from the one SCAD displayed and encoded in the organism, a diverse population of SCADs by varying the DNA sequence encoding the SCAD by mutation techniques. The new diverse SCADs generated in step 1010 are displayed on the surface of the organism (step 1020) and organisms are selected based on the surface expressed SCAD which bind to given antigens (step 1030). The organisms selected in step 1030 may be used in assays for the given antigen or may be further selected according to the binding or enzymatic characteristics of the gene product expressed on the surface.

Once any SCAD has been displayed on the surface of a microorganism, a large population of different SCADs can be generated by in vivo DNA synthesis, step 1010, and each cell or virion can display its own SCAD specificity, step 1020. Antigen binding to the displayed SCAD can be used to select those microorganisms harboring genes for SCADs which will bind antigen, step 1030. Once a strain of microorganisms is selected for antigen binding, it can be used as a sensitive assay for that antigen, step 1040. In step 1050, the ability to refine antigen binding is used to generate novel enzymes.

The steps needed to achieve the construction of a microorganism which displays SCAD are shown in the flow chart of Figure 2. In step 2000, a microorganism is selected. In step 2010, a gene within that organism is selected; the gene must be one coding for a protein which is displayed on the cell or virion surface. Preferably, the gene should not be essential to the organism. In step 2020, the gene for a SCAD to some known antigen is introduced into the selected gene, and in step 2030, this population of modified genes is put back into the organism. In step 2040 the genes are expressed, and in step 2050 the organisms are selected for binding to immobilized antigen. In step 2060, the gene is sequenced to determine which insertion was fruitful.

The steps needed to create a diverse population of SCADs displayed on the surface of a microorganism are illustrated in the flow chart on Figure 3. In step 3000, the Combining Determining Regions (CDRs) of the SCA are bounded by restriction sites. In step 3010, a large variety of DNA sequences are produced. Each sequence should begin with one of the restriction sequences and end with the corresponding restriction site. Between these sites should come any constant residues which are included to facilitate placement of restriction sites plus an integral number of triplets. The number of triplets can be varied within the bounds set by:

1. Analysis of sequences of natural antibodies with similar framework;
2. Computer modeling of the framework;
3. Trial and error.

In step 3020, these DNA sequences are inserted into the appropriate slots in the SCAD gene. In step 3030, these genes are reinserted into the organism and grown. The organism now contains a diversity of SCA specifications, each cell displaying its own particular SCAD. In step 3040, this population is passed over the inert support which will be used to support antigen. This step removes those organisms which bind to the inert support even without antigen.

In step 4000 in Figure 4, the antigen is attached to an inert support. In step 4010, the population of organisms prepared in steps 3000 to 3040 is passed over the supported antigen. Organisms not binding pass through. In step 4020, the organisms bound to the support are allowed to grow. In step 4030, colonies are found and sampled. In step 4040, the genes of several isolates are sequenced. In step 4050, the SCAD gene of selected organisms are mutagenized. In step 4060, step 4010 through 4050 are repeated with the mutagenized colonies. In step 4060, by washing more stringently, a SCA colony with maximal binding is obtained. Step 4060 can be repeated until suitable binding is obtained.

The present invention is also useful for the detection and quantification of known antigens. In step 5000 of Figure 5, a sample with unknown amount of an antigen is attached to an inert support. In step 5010, the strain of organism derived in step 4060 and displaying a SCAD against the antigen is passed over the inert support. In step 5020, the bound organisms are allowed to grow. In step 5030, points of growth are detected, the amount of growth quantitates the amount of antigen.

Enzymes, particularly degradative enzymes, work by stabilizing the transition state of a reaction. Ch mical theory suggests the shape of the transition states of many reactions. For example, the carbonyl carbons of esters of carboxylic acids are trigonal planar. The transition state for hydrolysis or tran-

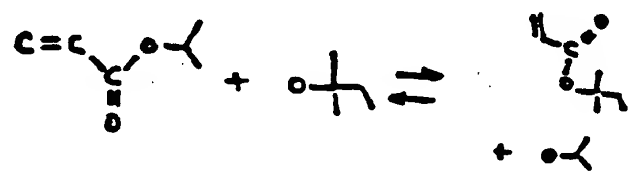
sesterification is almost certainly tetrahedral. It has recently been demonstrated that a monoclonal antibody against a phosphate ester (which is tetrahedral) is also an esterase.

Monoclonal antibody technology has many shortcomings for this task:

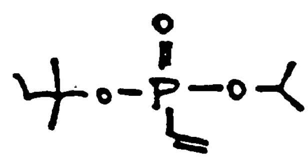
1. Slow turnaround
2. Difficulty in refining antibody
3. Inappropriate for highly toxic chemicals
4. Inappropriate for metabolites

The method described above can be applied to produce such enzyme-like binding molecules. It will be limited only in the ability to invent antigens which resemble the transition states of reactions wished to catalyze.

For example, to catalyse the transesterification



one can raise a SCA against



Having now generally described the invention, the same may further be understood by reference to the following expressly stated.

Example 1

The preferred embodiment utilizes the bacteriophage lambda. The gene V of lambda generates a protein which assembles to form the neck of lambda. First gene product V (gpV) forms hexameric annuli, then 32 of these annuli stack on the nose cone to form the neck (Figure 6). Finally, the neck joins the head which contains the DNA.

gpV is a protein of molecular weight 31K. Wild-type lambda have small protuberances on the outside of the neck annuli. Mutants have been isolated in which as much as 13K of gpV is absent. These mutants are viable, though temperature sensitive. The mutants are those wherein shortened gpV lack the protuberances on the neck annuli. Genetics indicates that the deletion is from the carboxy end of gene V.

SCAs made so far contain the four cysteine residues found in all V^H and V^1 domains of natural antibodies. Natural antibodies are secreted and fold in the oxidizing environment of serum. The interior of cells is a reducing environment; thus, one would not expect disulfide bonds to form. The sulfhydryl groups of cysteine lie only 2.0 A apart when a disulfide forms. If the disulfide is reduced, the sulfur atoms should lie 4.0 A apart. Thus, reduced cysteines will greatly destabilize folding of a SCA. Therefore, to get proper folding of SCAD inside a cell, one mutates the SCAD gene to change all or some of the CYS's to SER, THR, ALA, or GLY. In one embodiment, the SCA is against bovine growth hormone (BGH).

The V gene of lambda is shown in Figure 7. Genetics indicates that the domain responsible for the warts on the neck lies in the 300 to 400 last base pairs to the right. One cuts the gene at some point in this region, preferably 200 bases from the right end. A random number of bases on either side, up to 200 bases is removed. The SCAD (antiBGH) is inserted and put back into a lysogenic strain of E. coli. In the preferred embodiment, the lambda contains a highly beneficial gene for the E. coli.

The E. coli is induced. The lambda prog ny is passed over a support holding BGH. The E. coli is contacted with the support. The coli should be deficient in a way that the beneficial gene in the lambda will complement. For example the coli could be drug-sensitive and lambda will carry drug resistance. The corresponding antibiotic in the medium puts the coli under selective pressure so that only those cells

infected by lambda will grow. Only those lambda which bound antigen and stuck to the support are available.

Claims

1. An organism containing a recombinant gene wherein the product of the recombinant gene is a fusion of a polypeptide normally appearing on the outer surface of the organism with a single,chain antibody domain (SCAD), said SCAD being present on the outer surface of said organism.

2. An organism as claimed in claim 1 wherein the product of the recombinant gene comprises a polypeptide which directs the SCAD to the surface of the organism.

3. An organism as claimed.in claim 1 or 2 wherein the recombinant gene is non-essential.

4. An organism as claimed in claim 1, 2 or 3 wherein the SCAD possesses enzymatic activity.

5. An organism according to any preceding claim wherein the polypeptide normally appearing on the surface of the organism is an envelope protein.

6. A fusion polypeptide comprising a product normally appearing on the surface of an organism fused to a single chain antibody domain (SCAD).

7. A polypeptide as claimed in claim 6 wherein the SCAD possesses enzymatic activity.

8. A fusion polypeptide according to claim 6 or 7 wherein the product is an envelope protein.

9. A method of preparing an organism containing a single chain binding molecule on the outer surface of the organism which comprises:
   (1) isolating from an organism a first gene encoding for a cell polypeptide normally appearing on the surface of the organism;
   (2) inserting a second gene which encodes a single chain antibody domain into the first gene to form a recombinant fusion gene; and
   (3) transforming an organism with the recombinant fusion gene.

10. A method as claimed in claim 9 wherein the first gene is non-essential and/or the single chain binding molecule possesses enzymatic activity.

Patentansprüche

1. Organismus, welcher ein rekombinantes Gen enthält, wobei das Produkt des rekombinanten Gens eine Fusion eines normalerweise an der Außenfläche des Organismus aufscheinenden Polypeptids mit einer einzelkettigen Antikörperdomäne ("Single Chain Antibody Domain") (SCAD) ist und wobei die SCAD auf der Außenfläche des Organismus vorhanden ist.

2. Organismus nach Anspruch 1, worin das Produkt des rekombinanten Gens ein Polypeptid umfaßt, welches die SCAD zu der Oberfläche des Organismus richtet.

3. Organismus nach Anspruch 1 oder 2, worin das rekombinante Gen nichtessentiell ist.

4. Organismus nach Anspruch 1, 2 oder 3, worin die SCAD enzymatische Aktivität besitzt.

5. Organismus nach einem der vorangehenden Ansprüche, worin das Polypeptid, das normalerweise an der Oberfläche des Organismus aufscheint, ein Hüllprotein ist..

6. Fusionspolypeptid, welches ein Produkt enthältt, das normalerweise an der Oberfläche eines Organismus aufscheint und welches mit einer einzelkettigen Antikörperdomäne ("Single Chain Antibody Domain") (SCAD) verschmolzen ist.

7. Polypeptid nach Anspruch 6, worin die SCAD enzymatische Aktivität besitzt.

8. Fusionspolypeptid nach Anspruch 6 oder 7, worin das Produkt ein Hüllprotein ist.

9. Verfahren zur Herstellung eines Organismus, der ein Einzelketten-Bindungsmolekül an der Außenfläche des Organismus enthält, umfassend:
   (1) das Isolieren eines ersten Gens von einem Organismus, das für ein Zellpolypeptid kodiert, welches normalerweise an der Oberfläche des Organismus aufscheint;
   (2) das Einsetzen eines zweiten Gens, das für eine einzelkettige Antikörperdomäne ("Single Chain Antibody Domain") kodiert, in das erste Gen zur Bildung eines rekombinanten Fusionsgens;
   (3) die Transformation eines Organismus mit dem rekombinanten Fusionsgen.

10. Verfahren nach Anspruch 9, worin das erste Gen nichtessentiell ist und/oder das Einzelketten-Bindungsmolekül enzymatische Aktivität besitzt.

Revendications

1. Organisme contenant un gène recombiné, dans lequel le produit du gène recombiné est une fusion d'un polypeptide apparaissant normalement sur la surface extérieure de l'organisme avec un domaine d'anticorps à chaîne unique (SCAD), le SCAD étant présent sur la surface extérieure de l'organisme.

2. Organisme suivant la revendication 1, dans lequel le produit du gène recombiné comprend un polypeptide qui dirige le SCAD à la surface de l'organisme.

3. Organisme suivant la revendication 1 ou 2, dans lequel le gène recombiné est non essentiel.

4. Organisme suivant la revendication 1, 2 ou 3, dans lequel le SCAD possède une activité enzymatique.

5. Organisme suivant l'une quelconque des revendications précédentes, dans lequel le polypeptide apparaissant normalement sur la surface de l'organisme est une protéine d'enveloppe.

6. Polypeptide de fusion comprenant un produit apparaissant normalement sur la surface d'un organisme fusionné à un domaine d'anticorps à chaîne unique (SCAD).

7. Polypeptide suivant la revendication 6, dans lequel le SCAD possède une activité enzymatique.

8. Polypeptide de fusion suivant la revendication 6 ou 7, dans lequel le produit est une protéine d'enveloppe.

9. Procédé de préparation d'un organisme contenant une molécule fixant une chaîne unique sur la surface extérieure de l'organisme, qui comprend :
   (1) l'isolement d'un organisme, d'un premier gène codant pour un polypeptide cellulaire apparaissant normalement sur la surface de l'organisme;
   (2) l'insertion d'un deuxième gène qui code un domaine d'anticorps à chaîne unique dans le premier gène pour former un gène de fusion recombiné, et
   (3) la transformation d'un organisme avec le gène de fusion recombiné.

10. Procédé suivant la revendication 9, dans lequel le premier gène est non essentiel et/ou la molécule fixant une chaîne unique possède une activité enzymatique.

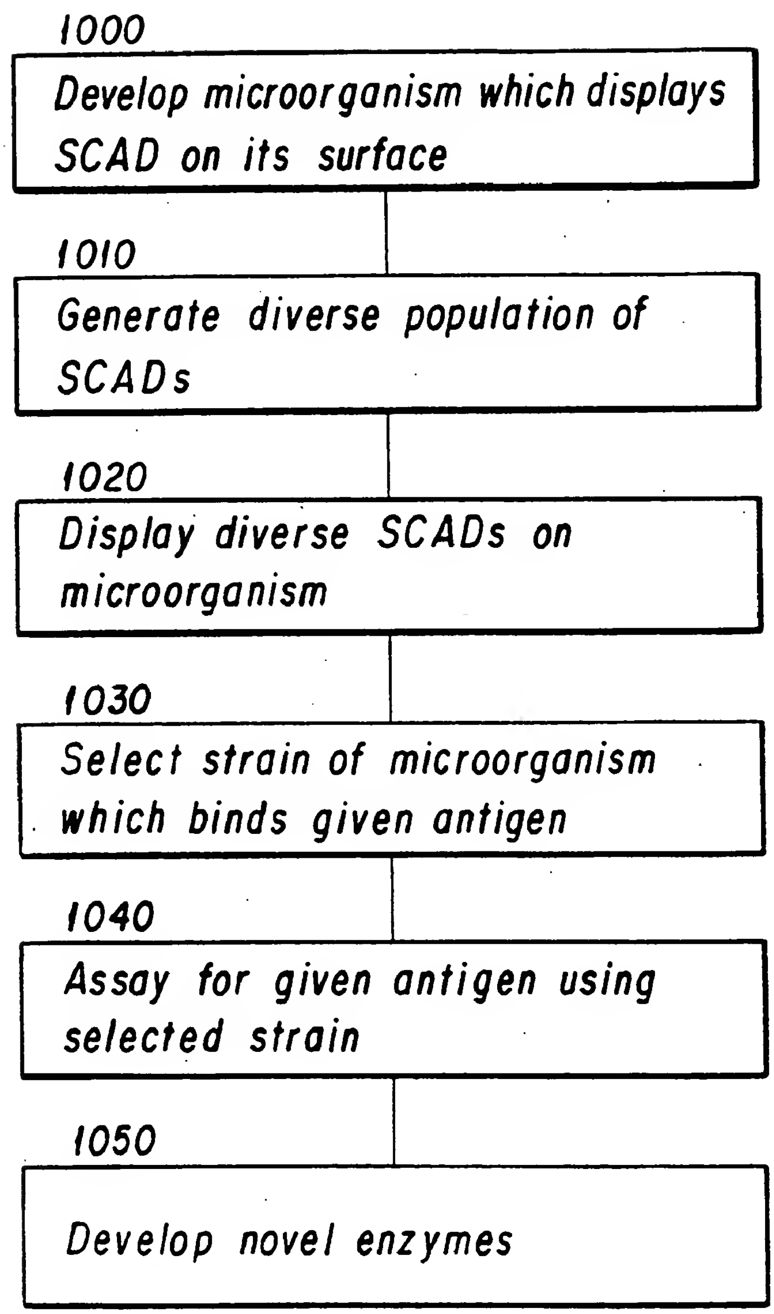

1000
Develop microorganism which displays SCAD on its surface
1010
Generate diverse population of SCADs
1020
Display diverse SCADs on microorganism
1030
Select strain of microorganism which binds given antigen
1040
Assay for given antigen using selected strain
1050
Develop novel enzymes

FIG. 1

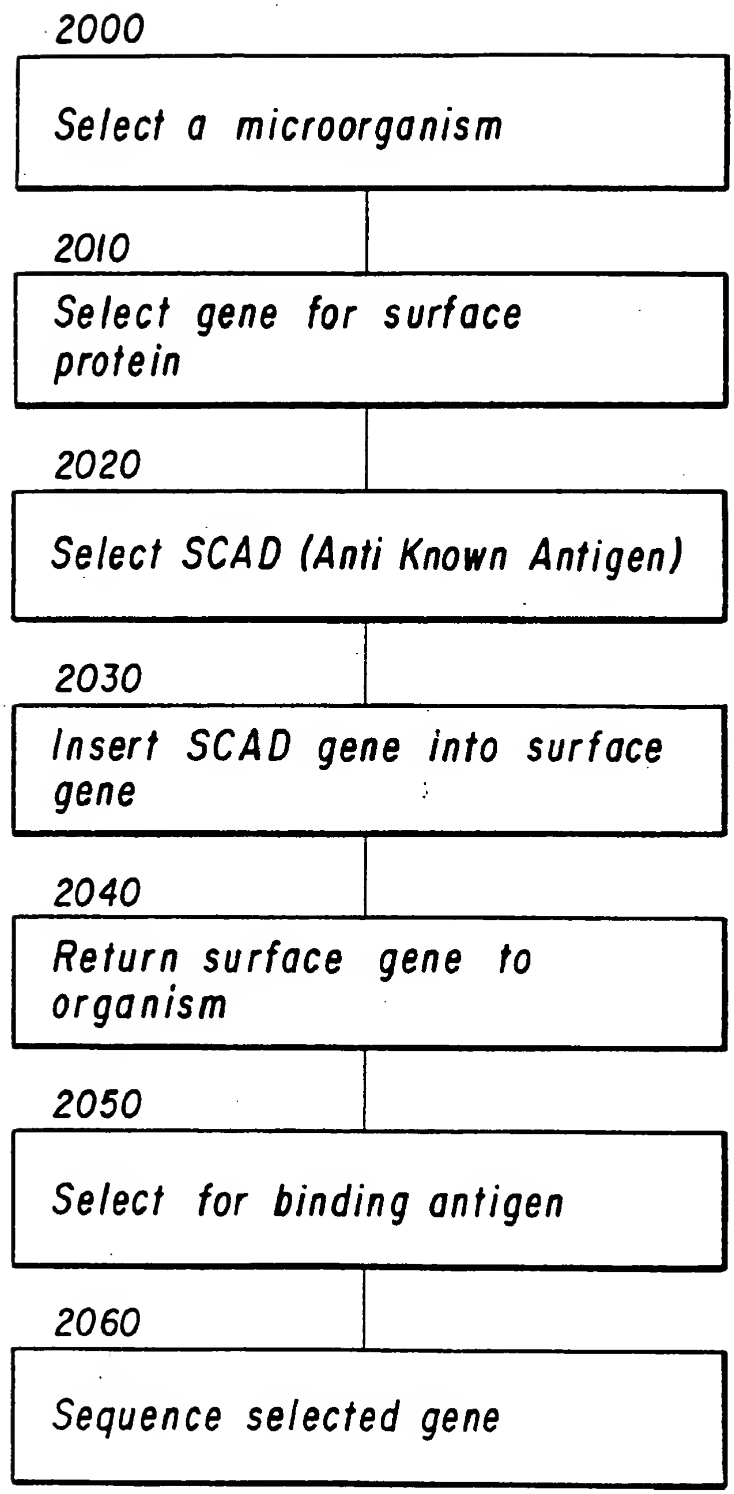

2000
Select a microorganism
2010
Select gene for surface protein
2020
Select SCAD (Anti Known Antigen)
2030
Insert SCAD gene into surface gene
2040
Return surface gene to organism
2050
Select for binding antigen
2060
Sequence selected gene

FIG. 2

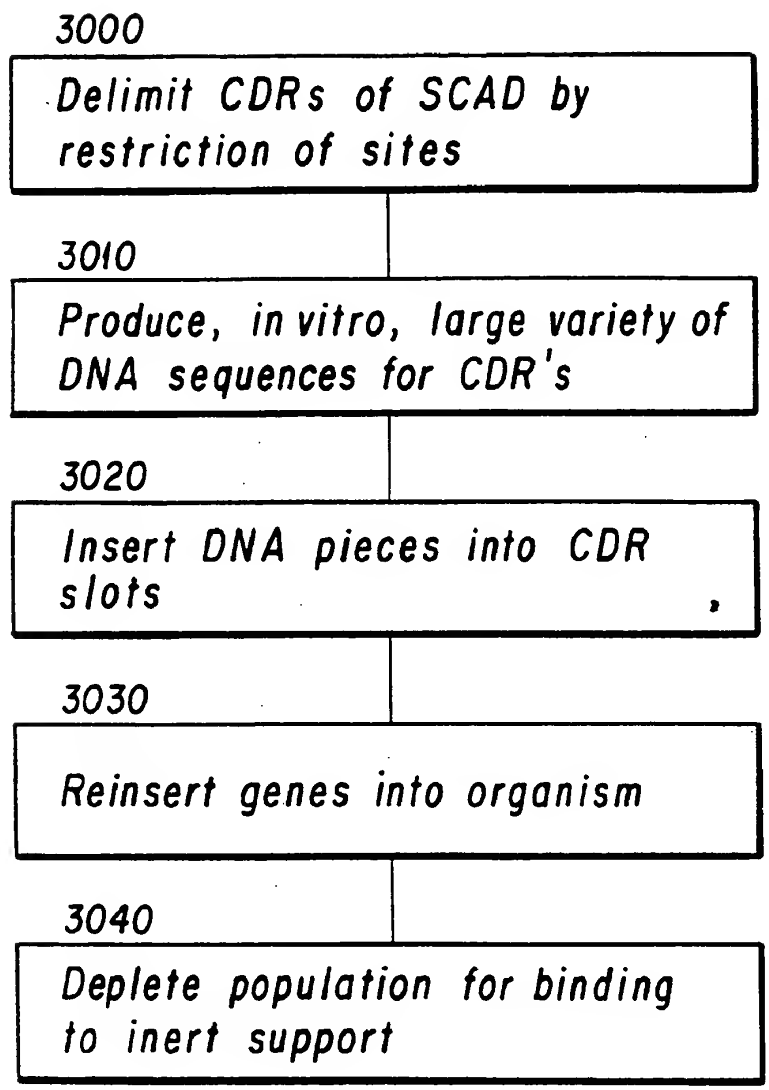

3000
Delimit CDRs of SCAD by restriction of sites
3010
Produce, in vitro, large variety of DNA sequences for CDR's
3020
Insert DNA pieces into CDR slots
3030
Reinsert genes into organism
3040
Deplete population for binding to inert support

FIG. 3

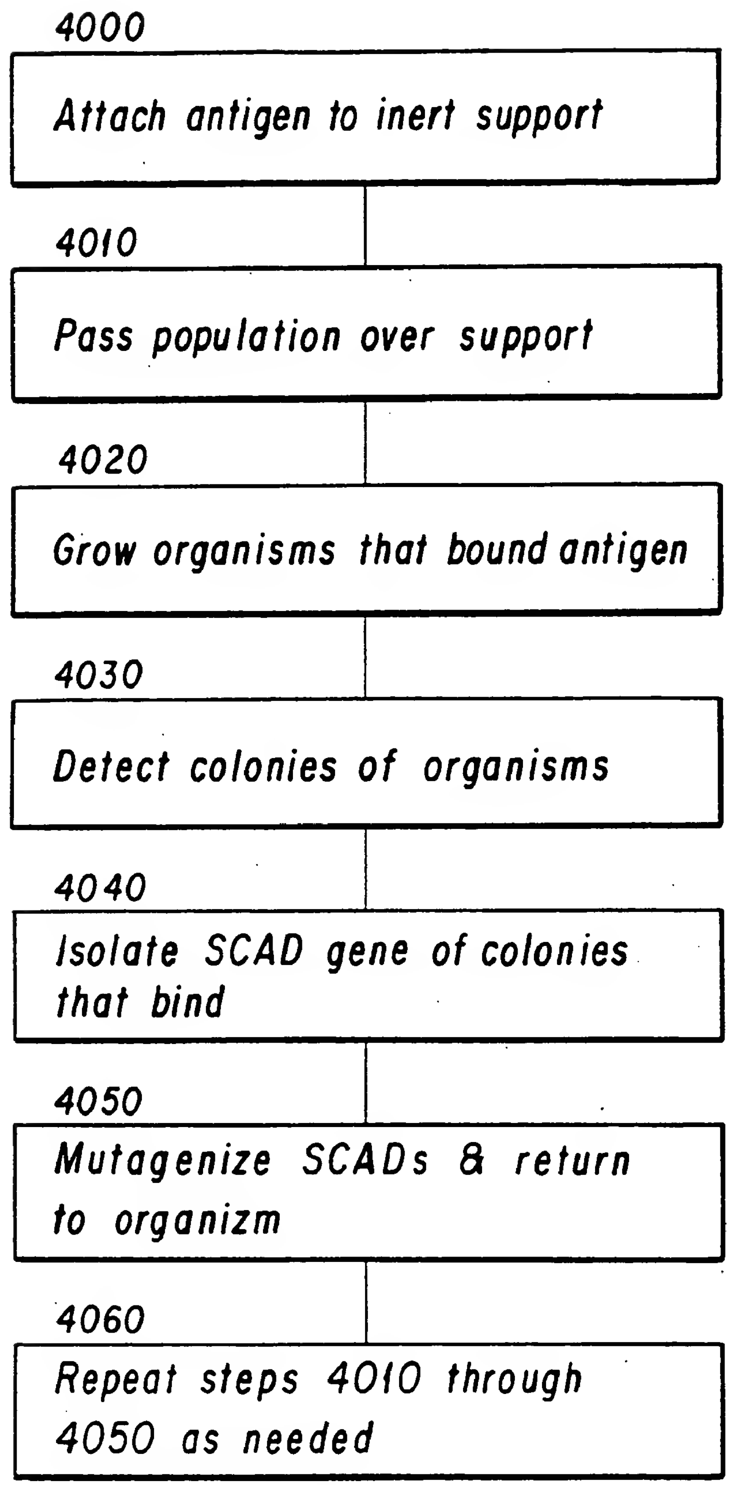

4000
Attach antigen to inert support
4010
Pass population over support
4020
Grow organisms that bound antigen
4030
Detect colonies of organisms
4040
Isolate SCAD gene of colonies that bind
4050
Mutagenize SCADs & return to organizm
4060
Repeat steps 4010 through 4050 as needed

FIG. 4

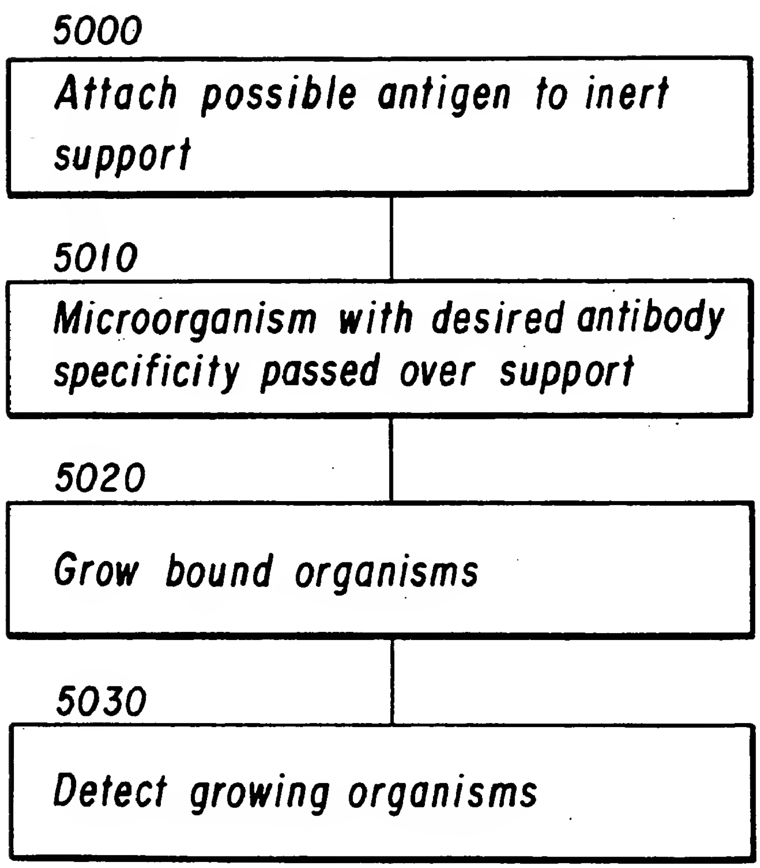


FIG. 5
Detecting Known Antigens

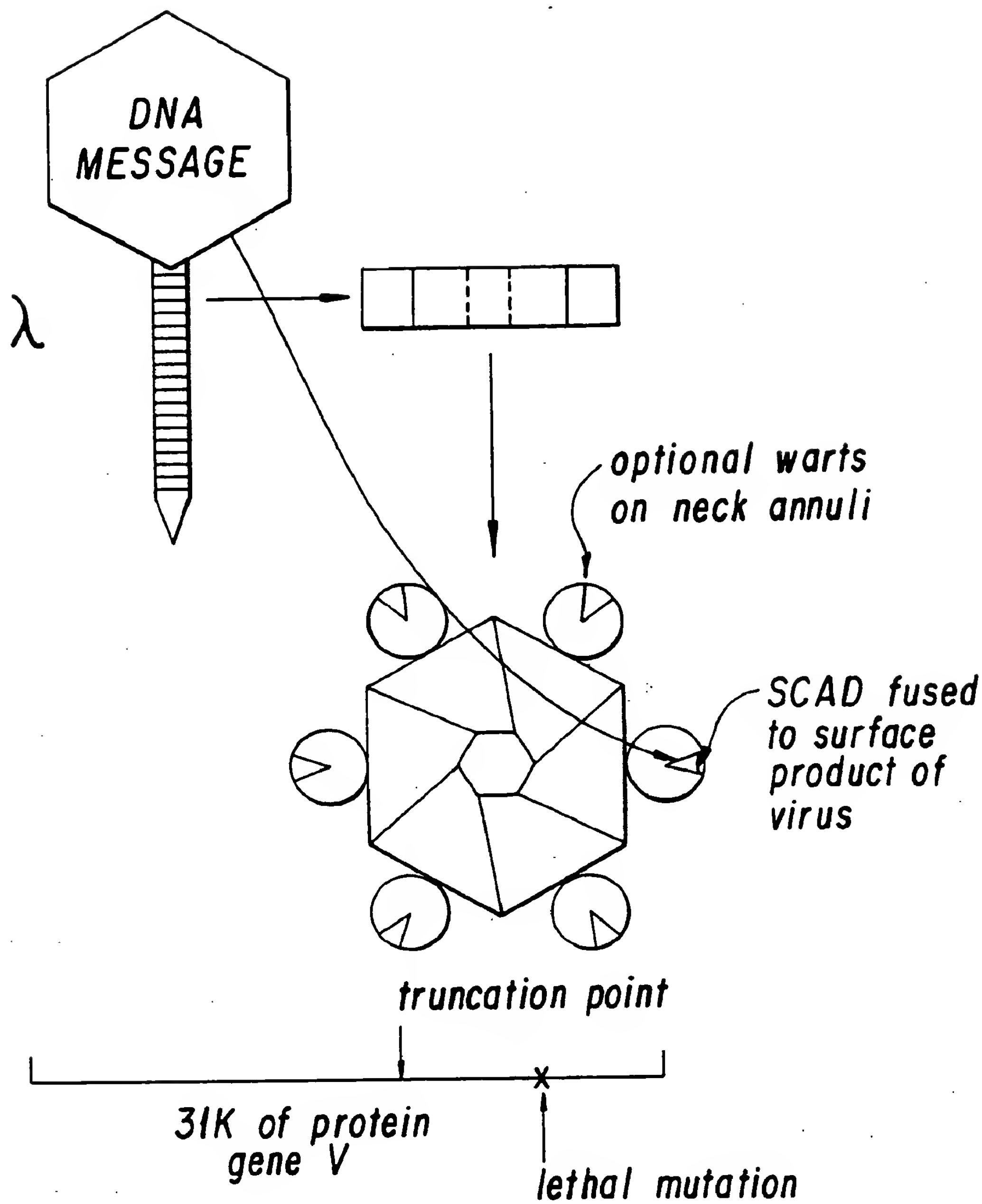

DNA
MESSAGE
λ
optional warts
on neck annuli
SCAD fused
to surface
product of
virus
truncation point
31K of protein
gene V
lethal mutation

FIG. 6

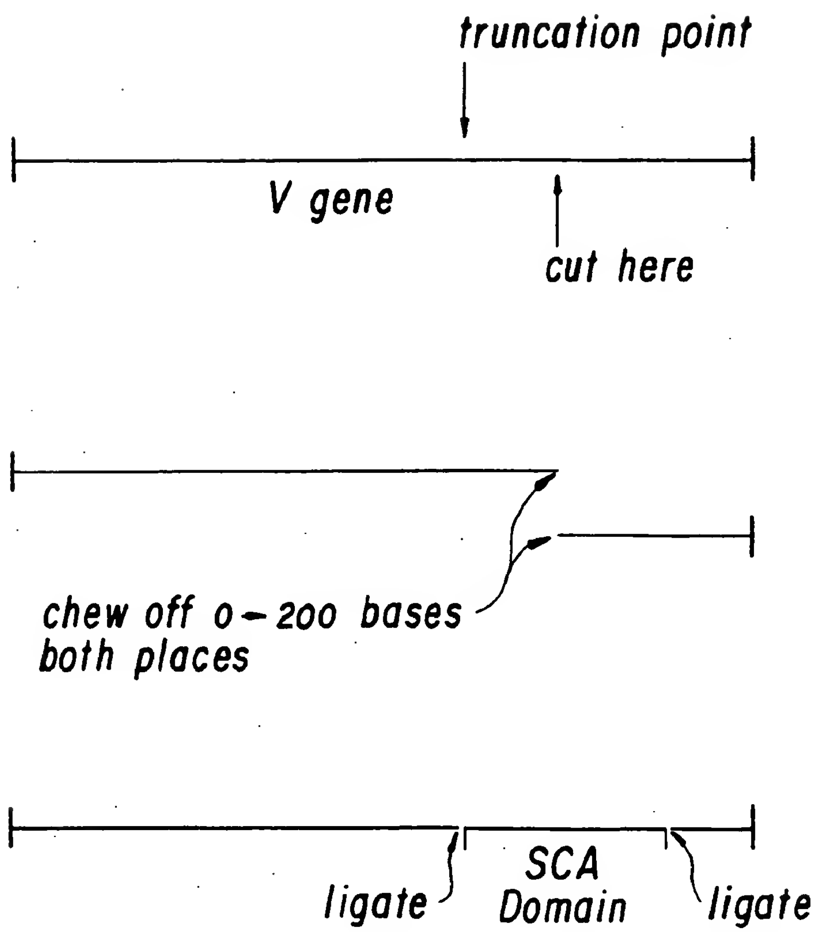

truncation point
V gene
cut here
chew off o – 2oo bases
both places
SCA
Domain
ligate
ligate

FIG. 7